
POPULATION GENETICS OF
THE CEDAR-GLADE
ENDEMIC *ASTRAGALUS*
BIBULLATUS (FABACEAE)
USING ISOZYMES¹

Carol J. Baskauf² and Sharon Snapp³

ABSTRACT

The rare cedar-glade endemic *Astragalus bibullatus* (Fabaceae) has low levels of genetic variability both within and among populations. Within-population means across 15 isozyme loci resulted in estimates of 1.4 alleles per locus, 25.6% polymorphic loci, and 0.064 for observed heterozygosity. Populations are genetically very similar, with a low F_{ST} and genetic identity values ranging from 0.981 to 1.000. Sites where this species naturally occurs should be protected, but, considering the low levels of population differentiation, the source of transplants or seeds needed to establish new populations may not be the most critical concern.

Astragalus bibullatus Barneby & E. L. Bridges (Fabaceae) is a rare plant endemic to the limestone ("cedar") glades of middle Tennessee's Central Basin. It is a perennial that overwinters as a rosette, flowers in April and May, and ripens fruits in June (Baskin & Baskin, 1989). *Astragalus bibullatus* was described as a new species in 1987, with the Great Plains taxon *A. crassicaarpus* Nutt. var. *crassicaarpus* considered to be its closest relative (Barneby & Bridges, 1987). Known from only a few sites, *A. bibullatus* is federally listed as endangered (FWS, 1991).

Conservation biologists are often concerned about levels of genetic variability present in rare species. Many authors have pointed out that species with little genetic variability would have limited evolutionary potential under heterogeneous or changing environments (e.g., Frankel, 1970, 1974; Franklin, 1980; Soulé, 1980; Beardmore, 1983; Bradshaw, 1984; Antonovics, 1984; Lande & Barrowclough, 1987; Hucenneke, 1991). Compared with more geographically widespread species, rare and localized species often (but not always) have low levels of genetic variability (Hamrick & Godt, 1990; Hamrick et al., 1991; Karro, 1987, 1991). Such low levels of genetic variability could be the result of inbreeding and/or random genetic drift in small populations (chronically small, or small due to founder events or other genetic

bottlenecks), or perhaps adaptation to a narrow set of environmental conditions.

When estimating the genetic diversity of a species, population genetic structure can be examined to evaluate the level at which most variability occurs (whether at the level of the individual, the population, or the entire species), and the genetic similarity of populations can be estimated. Such analyses can help in management decisions for rare species. For example, population "C" of *A. bibullatus* is located on private property the owner plans to bulldoze, so state conservation officials hoped to transplant most of these individuals to an *A. bibullatus* site on protected public land. Despite being protected, such a location could be unsuitable if the "C" population were genetically quite distinct from the resident plants. Possible negative effects could include reduced genetic diversity via local selection and random genetic drift, and poor growth of the transplants if genetic differences involve unique adaptations to local environmental conditions. Such possibilities raise the question of how genetically similar the *A. bibullatus* populations are.

This study examines the population genetic structure of *A. bibullatus*, using isozymes to estimate the genetic variability of this narrow endemic and the genetic similarity of its populations.

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² Department of Biology, Austin Peay State University, Clarksville, Tennessee 37044, U.S.A.

³ Austin Peay State University, Clarksville, Tennessee 37044, U.S.A. Present Address: University of Tennessee, Memphis, Tennessee 38163, U.S.A.

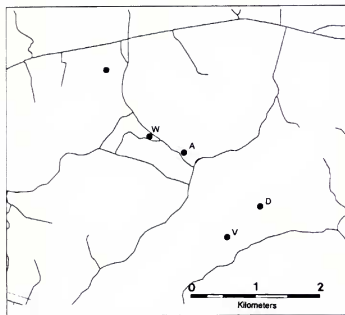


Figure 1. Relative positions of populations of *Astragalus bibullatus*. "W" represents both WO and WS. The unlabeled population was not sampled (see text). Population C (not shown) is about 20 km southwest of this area. Specific details about population locations have been omitted to protect this endangered species.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

Populations of *Astragalus bibullatus* were sampled during the summer of 1995. Leaves were collected and refrigerated in moist reclosable bags. Those collected in the field were stored on ice for a few hours before they were refrigerated.

Spatially separated clusters of plants are referred to here as "populations" and labeled specifically by letters (A, C, D, V, WO, and WS). Population C is relatively isolated, being about 20 km from the other populations; on the other hand, WO and WS are only about 70 m apart. All populations then known for the species were sampled except one—a possibly artificially established colony with perhaps 60–100 plants (FWS, 1991) to which the landowner refused access. At the time of sampling, population C existed almost entirely as potted plants in the greenhouse because individuals had been dug up for transplanting. Figure 1 shows the relative positions of all populations except for C, with "W" representing both WO and WS.

Virtually all plants present were sampled for C, D, and V. Individuals were sampled haphazardly from A, WO, and WS, with a large fraction of the plants present included in the sampling. The holotype for *Astragalus bibullatus* was collected from population A and is deposited in VDB (see Barneby & Bridges, 1987).

ELECTROPHORESIS

Electrophoresis procedures generally followed Werth (1985). Leaves were homogenized on ice in the simple extraction buffer to which 10% polyvinylpyrrolidone and 0.6% mercaptoethanol had been added immediately before grinding. Crude homogenate was then adsorbed onto filter paper wicks and loaded onto 12% starch gels. Various individuals were used as marker genotypes on gels throughout this study.

Four buffer systems (three continuous and one discontinuous) were used to visualize 12 enzyme systems:

- (1) tris-borate EDTA, pH 8, for alcohol dehydrogenase (ADH) (1.1.1.1), aldolase (ALD) (4.1.2.13), and glyceraldehyde-3-phosphate dehydrogenase (NAD-dependent form) (G3PDH) (1.2.1.12);
- (2) tris-citrate, pH 8, for isocitrate dehydrogenase (NADP-dependent form) (IDH) (1.1.1.42), malate dehydrogenase (MDH) (1.1.1.37), and phosphoglucosomerase (PGI) (5.3.1.9);
- (3) histidine-citrate, pH 5.7 (Wendel & Weeden, 1989), for menadiene reductase (MNR) (1.6.99.-), phosphoglucomutase (PGM) (5.4.2.2), phosphogluconate dehydrogenase (PGD) (1.1.1.44); and
- (4) the discontinuous system from Ridgeway et al. (1970), pH 8.1, for aspartate aminotransferase (AAT) (2.6.1.1), leucine aminopeptidase (LAP) (3.4.11.1), and triose-phosphate isomerase (TPI) (5.3.1.1).

Staining protocols generally followed Wendel & Weeden (1989). Other staining solutions are described in Werth (1985) (IDH), Moran & Hopper (1983) (MNR), Soltis et al. (1983) (G3PDH), and Baskauf (1993) (LAP, MDH, PGM). Loci and alleles were numbered from the electrophoretically fastest to the slowest.

ANALYSIS

Allele frequencies, measures of genetic variability, and Nei's (1978) unbiased genetic identity were calculated using BIOSYS-1 (Swofford & Selander, 1989); χ^2 goodness-of-fit tests of genotype frequencies for deviations from Hardy-Weinberg expectations (using the Levene correction for small samples) and χ^2 contingency tests to examine the independence of allele frequencies among populations were performed. Hierarchical cluster analysis (UPGMA) (Sneath & Sokal, 1973) was used to group populations by genetic similarity using Nei's genetic identity. Wright's (1978) F -statistics (F_{IS} and F_{ST}) for evaluating within vs. among population

Table 1. Allele frequencies and sample size (*N*) for polymorphic loci in *Astragalus bibullatus*.

Locus/allele	Population					
	A	C	D	V	WO	WS
PGM-1 (<i>N</i>)	32	28	21	16	22	18
1	0.203	0.000	0.024	0.000	0.045	0.028
2	0.016	0.107	0.048	0.063	0.045	0.139
3	0.781	0.893	0.929	0.938	0.909	0.833
PGM-2 (<i>N</i>)	32	28	21	16	22	18
1	0.859	0.839	0.929	0.875	0.364	0.833
2	0.063	0.036	0.000	0.000	0.091	0.111
3	0.078	0.125	0.071	0.125	0.545	0.056
ADH-1 (<i>N</i>)	30	24	16	8	21	15
1	0.367	0.188	0.250	0.063	0.262	0.233
2	0.633	0.813	0.750	0.938	0.738	0.767
PGD-2 (<i>N</i>)	32	21	21	15	22	16
1	0.984	1.000	0.976	0.967	0.841	0.938
2	0.016	0.000	0.024	0.033	0.159	0.063

variability were calculated according to Weir and Cockerham's (1984) procedures, which correct for effects of sample size and provide a weighting system for multiple alleles at a locus. *t*-tests were used to determine whether the value of an *F*-statistic differs significantly from zero.

RESULTS

Fifteen putative loci were considered to have been resolved, coding for only 10 of the enzyme systems. This is because interpretation was at least partially unclear for MDH, PGI, PGM, PGD, and TPI, usually due to the presence of a larger number of invariant bands than could be accounted for by the typical number of loci found in diploid plant species. Crossing studies are not helpful in such a case involving invariant loci, and comparisons of banding patterns of leaf tissue versus soaked pollen (Weeden & Gottlieb, 1980) did not aid interpretation. Liston (1992) reported duplication of certain isozyme loci (PGI, PGD, TPI, perhaps MDH) for some *Astragalus* taxa, and it is possible that there may be several cases of gene duplication in *A. bibullatus* as well.

Of the 15 loci resolved, 11 appear to be invariant for this species (ALD, LAP, AAT, IDH, PGD-3, MDH-3, the two G3PDH loci, and all three MNR loci). Allele frequencies for the four polymorphic loci are given in Table 1.

Astragalus bibullatus does show some genetic variability for soluble enzymes, but at a low level (Table 2). Within populations, 20% to 27% of the loci included in this analysis are polymorphic (*P*). The mean number of alleles per locus (*A*) is 1.4.

Observed heterozygosity (*H_o*) for these loci ranges from 0.038 (for V) to 0.099 (for WO), with a mean of 0.064. Species level estimates are similar, with *A* = 1.4 and *P* = 27%. These estimates of isozyme variability may be overestimates, considering that some unknown number of clearly invariant loci were excluded from the analysis.

As a whole, the populations are somewhat differentiated from one another at three of the four variable loci, as indicated by the significant (*P* < 0.01) χ^2 contingency tests of allele frequencies (Table 3). Further analysis revealed that despite being separated by only about 70 m, WO and WS show highly significant differences (*P* < 0.001) in allele frequencies at PGM-2. In fact, WO appears to be genetically the most distinctive population in the species.

Nonetheless, the populations of *A. bibullatus* are all very similar genetically. Genetic identity values among these populations are consistently high, ranging from 0.981 to 1.000 (Table 4, Fig. 2). An *F_{ST}* of 0.089 (Table 5) indicates that less than 10% of the total genetic variability of the species is the result of differences among populations, and in fact the jackknifed mean *F_{ST}* does not differ significantly from zero (*P* > 0.05). Therefore, most variability in this species is due to genetic heterogeneity within populations rather than genetic differentiation among populations.

Genotype frequencies for variable loci do not deviate significantly from the Hardy-Weinberg expectations within populations; thus expected heterozygosity values (*H_e*) are very close to observed values (*H_o*) for this species (Table 2). This situation

Table 2. Genetic variability* at 15 loci for *Astragalus bibullatus*.

Population	N	A	P	H _o	H _e
A	29.3 (0.7)	1.4 (0.2)	26.7	0.064 (0.034)	0.074 (0.040)
C	26.1 (0.7)	1.3 (0.2)	20.0	0.061 (0.033)	0.053 (0.029)
D	20.4 (0.4)	1.3 (0.2)	26.7	0.056 (0.034)	0.047 (0.027)
V	15.8 (0.8)	1.3 (0.1)	26.7	0.038 (0.019)	0.036 (0.018)
WO	21.3 (0.4)	1.4 (0.2)	26.7	0.099 (0.049)	0.095 (0.047)
WS	14.0 (1.0)	1.4 (0.2)	26.7	0.068 (0.032)	0.072 (0.034)
Mean (all populations)		1.4	25.6	0.064	0.063

* Mean sample size per locus (N), mean number of alleles per locus (A), percentage of loci polymorphic (P), observed heterozygosity (H_o), expected heterozygosity (H_e) as an unbiased estimate (Nei, 1978). Standard errors are indicated in parentheses.

is reflected in the fact that F_{IS} values are close to zero and the jackknifed mean does not differ significantly from zero (Table 5). These data suggest that *A. bibullatus* may be primarily an outcrossing species; however, this species' mating system has not been studied.

DISCUSSION

Although not completely lacking in genetic diversity at isozyme loci, the narrow endemic *Astragalus bibullatus* has low levels of variability. This is true for each population and for the species as a whole. In a compilation of plant isozyme studies, Hamrick and Godt (1990) reported population level means of $A = 1.72$, $P = 43.0\%$, and $H_e = 0.159$ for 85 studies of widespread species, as opposed to $A = 1.39$, $P = 26.3\%$, and $H_e = 0.063$ for 100 studies of narrowly endemic species. Thus means estimated for the rare *A. bibullatus* ($A = 1.4$, $P = 25.6\%$, and $H_e = 0.063$) are comparable to those given for endemics in general. Low levels of variability at isozyme loci also have been reported for

some western species of *Astragalus* with restricted geographic ranges (Karron, 1991; Liston, 1992). On the other hand, Travis et al. (1996) found 220 variable AFLP markers and substantial differentiation among populations for the rare *Astragalus cremnophylax* Barneby var. *cremnophylax*. These data are not directly comparable to isozyme data, however, and it is not known what levels of diversity or population differentiation would be detected by an isozyme survey of this taxon.

A few other species endemic or nearly endemic to the limestone glades of Tennessee have been assayed for variability at isozyme loci. Levels of genetic variability estimated for *Echinacea tennesseensis* (Beadle) Small (Asteraceae), another federally listed endangered species, were similar to those of *A. bibullatus*: $A = 1.3$, $P = 23.0\%$, and $H_e = 0.071$ (Baskauf et al., 1994). A much less rare congener, *Astragalus tennesseensis* A. Gray, had relatively high estimates, with $A = 1.71$, $P = 43.1\%$, and $H_e = 0.148$ (calculated from Wiltshire,

Table 4. Genetic identities (Nei, 1978): pairwise comparisons for populations of *Astragalus bibullatus*.

Locus	# of alleles	DF	χ^2	P
PGM-1	3	10	36.263	0.00008
PGM-2	3	10	67.705	0.00000
ADH-1	2	5	8.312	0.13986
PGD-2	2	5	16.539	0.00546

Table 3. Independence of allele frequencies for populations of *Astragalus bibullatus*: χ^2 contingency analyses.

Population	A	C	D	V	WO	WS
A	*****					
C	0.997	*****				
D	0.998	1.000	*****			
V	0.992	1.000	0.999	*****		
WO	0.981	0.985	0.981	0.982	*****	
WS	0.999	1.000	1.000	0.999	0.984	*****

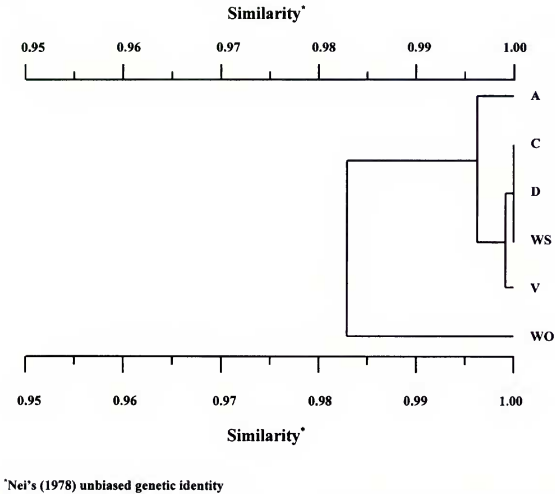


Figure 2. Populations of *Astragalus bibullatus* clustered according to genetic similarity. The similarity measure used is Nei's (1978) unbiased genetic identity.

1994). However, *Dalea foliosa* (A. Gray) Barneby, another legume federally listed as endangered, has much lower estimates, with $A = 1.15$, $P = 13.8\%$, and $H_e = 0.045$ (calculated from Wiltshire, 1994).

Population sizes of *Astragalus bibullatus* appear to be quite variable among years and have been extremely small at times (Somers & Gunn, 1990; FWS, 1991), a factor that could contribute to low levels of genetic variability. For example, the "A" population was reported to consist of only a couple plants in 1979, but had increased to 171 plants by

1988 after the site had been cleared of woody vegetation. Such dramatic population fluctuations or extinction and recolonization events, even when rare, can greatly decrease effective population sizes and thus genetic variability (Wright, 1940; Nei et al., 1975; Lande & Barrowclough, 1987; McCauley, 1993). Such population crashes have been observed for some species showing no genetic variability at isozyme loci (Lesica et al., 1988; Waller et al., 1987), as well as some *Astragalus* species showing very low levels of variability (e.g., *A. clarianus* Jepson; Liston, 1992).

"Genetic bottlenecks" resulting from population crashes are not the only factor that could affect genetic variability in *A. bibullatus*. Even at the best of times populations of this species are not large, and the smaller a population the more quickly random genetic drift is likely to erode variability. On the other hand, this plant is a perennial that probably has a long-term seed bank like many of its congeners (e.g., *A. tennesseensis*; Baskin & Baskin, 1989). Both of these features would favor the retention of genetic variability within the species.

The fine-scale differentiation observed between "populations" WO and WS, which are separated by only 70 m, was unexpected considering the great

Table 5. F -statistics for polymorphic loci in *Astragalus bibullatus*.

Locus	F_{IS}	F_{ST}
PGM-1	-0.108	0.041
PGM-2	0.041	0.194
ADH-1	-0.029	0.019
PGD-2	0.090	0.053
Mean*	-0.009 NS (0.029)	0.089 NS (0.060)

* Means jackknifed over polymorphic loci (Weir & Cockerham, 1984), with standard errors indicated in parentheses. Neither mean differs significantly from zero (NS, $P > 0.05$).

similarity among populations as a whole for this species. WO is also the population that displays the highest levels of heterozygosity. Of all populations, WO occurs in the most open habitat—a regularly mowed area along a private lane. The WS plants, on the other hand, grow in one of the most shaded spots among trees. Our isozyme data suggest that limited gene flow occurs between these two populations despite their close proximity. Gene flow could be restricted due to pollinator behavior, or could be ineffective due to differential selection pressures.

The most immediate threat to survival for *A. bibullatus* appears to be lack of protected habitat, with all populations but two occurring on privately owned land. The plight of the "C" site is a clear indication of this threat. WO could be a particularly good population to try to protect, given that it is genetically the most distinctive (indicated by genetic identity values) and the most variable (indicated by heterozygosity estimates); nevertheless, all of the populations are genetically quite similar. Extinction because of environmental stochasticity is a risk for any highly localized species limited to a few populations (Lande, 1988; Simberloff, 1988); thus the establishment of new populations of this species is advisable. A seed storage program is already in progress (K. Havens, pers. comm.). The low level of population differentiation observed for *A. bibullatus* suggests that the origin of seed used in establishing new populations probably is not a critical consideration. Similarly, these data provide no evidence of major genetic differences that might make inadvisable the transplanting of individuals from C to the V population.

Overall, it appears that protection of natural populations and the establishment of new populations are high priorities in alleviating the threat of extinction for this rare species. In addition, further research is needed. Little is known about the life cycle and ecology of *Astragalus bibullatus*, and any management plans would benefit from this type of information. Furthermore, it would be interesting to know how the genetic variability of this cedar-glade endemic compares with that of its widespread prairie relative, *A. crassicaarpus* var. *crassicaarpus*, and such a comparison is planned.

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